Note

Molecular mapping of the brace root traits in sorghum (*Sorghum bicolor* L. Moench)

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The presence and morphology of plant brace roots are important root architecture traits. Brace roots contribute significantly to effective anchorage and water and nutrient uptake during late growth and development, and more importantly, have a substantial influence on grain yield under soil flooding or water limited conditions. However, little is known about the genetic mechanisms that underlie brace root traits. In this study, quantitative trait loci (QTLs) for presence of brace roots from the sorghum landrace "Sansui" were mapped and associated molecular markers were identified. A linkage map was constructed with 109 assigned simple sequence repeat markers using a F_2 mapping population derived from the cross Sansui/Jiliang 2. Two QTLs associated with presence of brace roots were localized on chromosomes 6 and 7. The major QTL on chromosome 7 between markers Dsenhsbm7 and Xcup 70 explained about 52.5% of the phenotypic variation, and the minor QTL on chromosome 6 was flanked by Xtxp127 and Xtxp6 and accounted for 7.0% of phenotypic variation. These results will provide information for the improvement of sorghum root architecture associated with brace roots.

Key Words: sorghum, brace roots, SSR, QTL mapping.

Introduction

Plant root system architecture is of great agronomic importance because it is a key determinant for plant anchoring and mechanical support, propagation, storage, and water and nutrient uptake, and as the major interface between the plant and various biotic and abiotic factors (De Smet *et al.* 2012, Orman-Ligeza *et al.* 2013). Therefore, plant root system architecture has emerged in recent years as the central focus for plant biology study. Higher plants usually have a complex root system, including embryonic primary roots, seminal roots, lateral roots and shoot borne roots, which form at different developmental stages and have distinct physiological functions (Hochholdinger *et al.* 2004a, 2004b). Specifically, the sorghum root system consists of three types of roots: primary roots, secondary or adventitious roots and brace or buttress roots. Primary roots develop from the radicle and die subsequently. Secondary or adventitious roots develop from the first node from the mesocotyle and from the second internode and above. These roots are branched laterally and mainly supply nutrients to the plant. Brace or

buttress roots develop from the root primordia of the basal nodes above the ground level. These roots differ from the main roots in that they are mostly very short, lose their meristem and rapidly become determinate (Varney and McCully 1991). They are the most important root architecture traits influencing plant body stability and lodged plant restoration, which limit high density planting systems to reach greater yield. Studies have demonstrated that in maize, brace roots contribute enormously to lodging resistance, and water and nutrient uptake during late growth and development of plants (Varney and Canny 1993, Wang *et al.* 1994). Most importantly, they have a substantial influence on grain yield under soil flooding and water-limited conditions (Hochholdinger and Tuberosa 2009).

Previous studies on brace roots have been focused mainly at the morphological and physiological levels. Primordia of brace roots develop from dedifferentiated cells of the stem parenchyma, just behind the stem cortex and below the intercalary meristem of the overlaying internodes (Hoppe *et al.* 1986). Nutritional factors like phosphorus deficiency (Pellerin 1994) and nitrogen deficiency (Pellerin *et al.* 2000) or environmental factors like low light intensity (Demotes-Mainard and Pellerin 1992, Pellerin 1994) and soil ridging (Thomas and Kaspar 1995, 1997) have been shown to affect brace root formation. Several studies have started to exploit the brace root initiation or early development at the

molecular level, and some genes possibly involved in regulation of the lateral or adventitious root formation and brace root formation have been identified in Arabidopsis (Himanen *et al.* 2004), rice (Inukai *et al.* 2005), and maize (Husakova *et al.* 2013, Li *et al.* 2011, Muthreich *et al.* 2013). However, the genetic mechanisms that underlie brace root traits remain poorly understood. Some studies have tried to detect QTLs controlling root traits, such as root growth angle, seminal and lateral root length and number and their contributions to grain yield and drought adaptation in maize (Cai *et al.* 2012), wheat (Christopher *et al.* 2013) and sorghum (Mace *et al.* 2012, Rajkumar *et al.* 2013, Singh *et al.* 2012). For brace root characterization in maize, Landi *et al.* (2002) identified the QTL associated with the number of brace roots, and recently, QTLs controlling total brace root tier number and effective brace root tier number were identified from two mapping populations (Ku *et al.* 2012). These results may provide useful information for understanding the molecular mechanisms controlling root architecture.

To date, there have been no reports on gene mapping of brace root formation in sorghum. The objectives of this study were to characterize the brace root inheritance pattern in sorghum and identify the controlling locus for the presence of brace roots using a F_2 population. The results will provide information for the improvement of sorghum root architecture associated with brace roots.

Materials and Methods

Plant materials and phenotypic evaluation

The maternal line, Sansui, was a Chinese landrace traditionally used as a broomcorn with brace roots on the basal 6–8 nodes. Its plant height was 430 cm with 12–14 internodes. The paternal line, Jiliang 2, was an elite sorghum cultivar widely used for grain production in North China with very few brace roots. Its plant height was 151 cm with 13–15 internodes. For mapping population construction, Sansui was crossed as maternal parent with Jiliang 2. The plant height of their F_1 was 503 cm with 20–24 internodes. A total of 611 F_2 progenies, together with 20 each parental lines and 10 F_1 individuals, were planted at Shijiazhuang experimental station (38°04′N 114°29′E) in May 2011. The planting density was 40 cm rows × 20 cm between plants. Field management was in accordance with local practices. When the plants were mature, 219 consecutive plants from the middle of the experimental plot were sampled for evaluation of brace roots.

DNA extraction and simple sequence repeat (SSR) marker analysis

Total genomic DNA was extracted from young leaves using the CTAB method (Murray and Thompson 1980). To obtain polymorphic SSR markers between Sansui and Jiliang 2, SSR markers covering the whole sorghum genome were first surveyed with the two parental lines. The infor-

mative SSR markers identified by this screening were then used for genotyping of the F_2 individuals. Information on some of the SSR markers used in this study is displayed in a supplemental file (Supplemental Table 1). PCR was performed with 50 ng genomic DNA, 100 ng primer pair, 125 μM dNTP, 50 mM KCl and 10 mM Tris-HCl, 2 mM MgCl$_2$, and 1 unit Taq polymerase. The amplification procedure consisted of one cycle at 94°C for 3 min, followed by 35 cycles of 1 min at 94°C, 1 min at 55 to 58°C depending on the primer pair, 1 min at 72°C, and a final extension step at 72°C for 8 min. The PCR products were separated on a 5% polyacrylamide gel followed by silver staining.

### Data analysis, linkage map construction and QTL detection

To characterize the relationship between brace roots and other phenotypic traits, such as plant height, stem diameter, flag leaf length, internode number, spike node length and spike length, univariate and nonparametric bivariate correlations were performed using the Pearson correlation coefficient. Statistical significance was assumed when a null hypothesis could be rejected at $P < 0.05$ and 0.01. Statistical analysis was performed using SPSS 11.5 for Windows. The mapping data was analyzed using MAPMAKER/EXP version 3.0b (Lincoln *et al.* 1993) using the Kosambi map function to calculate genetic distances. Linkage was determined at the logarithm of odd (LOD) threshold of 3.0 with a maximum map distance of 50 centiMorgan (cM). The map positions of the markers were visualized using the software Windows QTL IciMapping version 3.2 (http://www. isbreeding.net).

QTL analysis to detect main effect QTL was conducted by using Windows QTL IciMapping version 3.2 following the inclusive composite interval mapping of additive (ICIM-ADD) module within the software. Regions with a LOD score above 3.5 were considered as suggestive of a QTL. Additive QTL was detected using a 1.0 cM step in scanning. The probability used in stepwise regression was 0.001. Threshold LOD scores for detection of definitive QTL were also calculated based on 1000-permutations. Type I error rate to determine the LOD threshold from permutation tests was 0.05 (Sun *et al.* 2013).

Results

Characterization of brace root traits in Sansui, Jiliang2 and their offspring

Brace roots were observed around the basal 6–8 nodes of Sansui and F_1, while brace roots were found only around the first node of Jiliang 2 (Fig. 1A). The F_1 plants had shorter brace roots with a different morphology from those seen on Sansui nodes. The higher number of nodes in F_1 plants was similar to that of Jiliang 2, although brace root numbers in F_1 plants were similar to those of Sansui. Most of the brace roots at upper nodes were aerial roots, which could have played a role in lodged plants. There was significant brace root variation in different F_2 plants (Fig. 1B). The brace
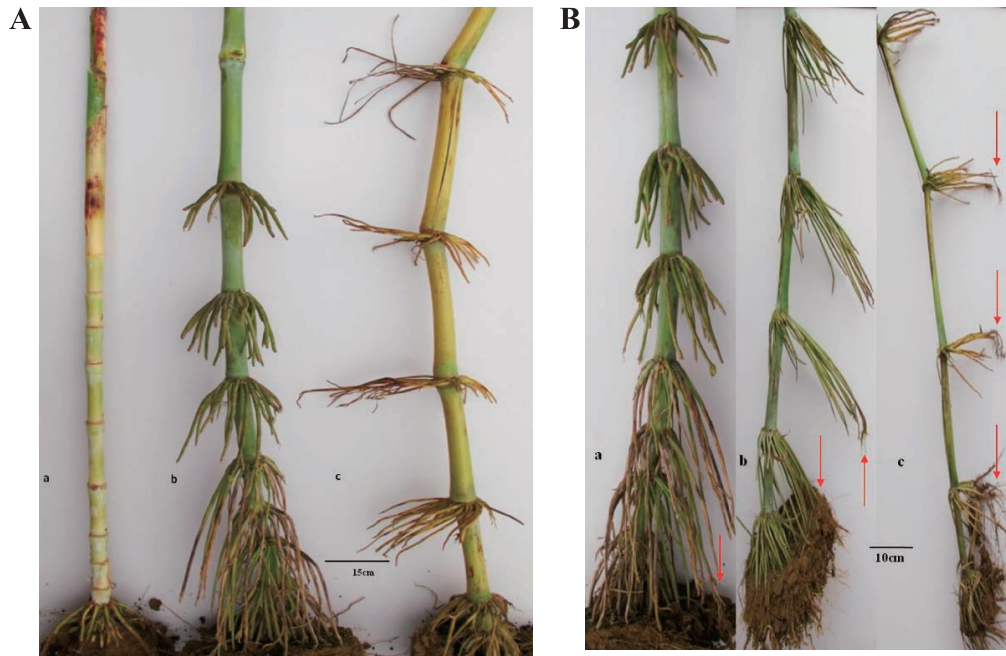
Fig. 1. A: A diagram showing the presence of brace roots on (a) Jiliang 2, (b) Sansui/Jiliang 2 F_1 and (c) Sansui. B: The brace roots at basal nodes provide plant body anchorage (arrowed) under normal growth condition (a), and upper node roots provide support and nutrient and water uptake (arrowed) under slightly lodged (b) and heavily lodged conditions (c).

roots at basal nodes contributed to anchoring the plant body to the ground under normal growth condition (Fig. 1B a), and upper node roots, on coming in contact with the soil, provided above ground support to help the lodged plant recover by "kneeing up" and nutrient and water uptake (Fig. 1B b, c). The average numbers of nodes with brace roots of Sansui, Jiliang 2 and their F_1 were 6.2 ± 0.89 (n = 20), 1.25 ± 0.44 (n = 20) and 6.4 ± 0.84 (n = 10), respectively. The average number of nodes with brace roots in the F_2 mapping population was 4.11 ± 2.98 (n = 291). Correlation analysis showed that number of nodes with brace roots was significantly correlated with other plant traits, including plant height (r = 0.649), stem diameter (r = 0.425), flag leaf length (r = 0.206), internode number (r = 0.213) and spike length (r = 0.143) (Table 1). A negative correlation was, however, observed in number of nodes with brace roots and spike node length (r = –0.343).

Linkage map and QTLs

A total of 326 simple sequence repeat (SSR) markers selected according to their uniform distribution throughout 10 chromosomes of sorghum were used to initially screen polymorphisms between Sansui and Jiliang 2. Among them, 141 markers were polymorphic between the two parents. After screening the F_2 population with the polymorphic SSR markers, the genotype and phenotype data were analyzed by employing the MAPMAKER program. Finally a total of 109 markers (77.3%) were assigned to 12 linkage groups representing all 10 sorghum chromosomes and covering a total distance of 1,114.5 cM with an average interval length of 10.2 cM.

Table 1. Pearson correlation coefficients for brace root and agronomic traits including number of nodes with brace roots (BR), plant height (PH), stem diameter (SD), flag leaf length (FLL), internode number (IN), spike node length (SNL) and spike length (SL)

|	PH	SD	FLL	IN	SNL	SL
BR	0.649**	0.425**	0.206*	0.219**	–0.343**	0.143*
PH		0.537**	0.363**	0.202**	–0.163**	0.383**
SD			0.378**	0.046	–0.100	0.322**
FLL				–0.109	0.071	0.490**
IN					–0.125*	–0.005
SNL						0.083

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 2. Chromosome (Chr.) locations, peak positions (cM), flanking markers, LOD scores, phenotypic variations explained (PVE), additive (ADD) and dominant effects of quantitative trait loci (QTLs) detected for presence of brace root using "Sansui/Jiliang 2" F_2 population

QTL	Chr.	Peak position(cM)	Flanking markers	LOD	PVE (%)	ADD
qRT6	6	97	Xtxp127-Xtxp6	9.1	7.0	1.2
qRT7	7	79	Dsenhsbm7-Xcup70	47.8	52.5	3.1

Two QTLs associated with the presence of brace roots were identified by using the ICIM mapping program (Table 2). These were located on chromosomes 6 and 7 with the "Sansui" alleles increasing number of nodes with brace roots. The major QTL on chromosome 7, named "*qRT7*", was mapped between markers Dsenhsbm7 and Xcup70 at 20.1 cM apart (Fig. 2). This QTL explained 52.5% of the phenotypic variation with LOD score of 47.8. The second
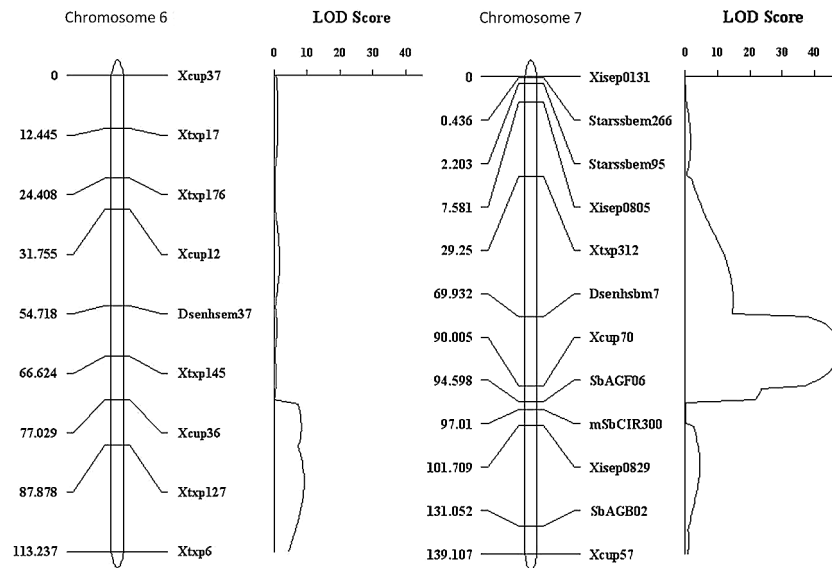
Fig. 2. Linkage map of the region harboring two quantitative trait loci for brace roots on chromosome 6 (left) and 7 (right) in the "Sansui/Jiliang 2" F_2 mapping population. The genetic distances (cM) between adjacent markers are shown on the left of each chromosome, whereas the names of mapped markers are on the right. The Y-axis of QTL map is LOD score and X-axis is map distance as indicated in the linkage maps. *qRT6* was positioned between markers Xtxp127 and Xtxp6; *qRT7* was between markers Dsenhsbm7 and Xcup70.

QTL on chromosome 6, named "*qRT6*", accounted for 7% of the phenotypic variation with LOD value of 9.1. This QTL was located in the marker interval Xtxp127-Xtxp6, spanning about 25.4 cM. The two QTLs together explained 59.5 % of the phenotypic variation.

Discussion

Two QTLs associated with sorghum brace roots have been identified in the present study which explained most of the phenotypic variation, indicating that two major genes control the presence of brace roots in sorghum landrace "Sansui". The QTL on chromosome 7, *qRT7*, was clearly the most important locus determining the trait variation. Initially, we treated the trait as a single dominant gene; however, despite the existence of its large effect, an additional locus has been found through the QTL scanning, which suggests that a more complicated genetic mechanism controlled the trait.

Brace roots are key to sorghum's stand ability. A previous study on sorghum showed that lodging resistance was associated with larger diameters of basal internodes and peduncles, shorter peduncles, shorter plant height, higher weight of 5-cm basal and peduncle stalk sections, and a thicker rind (Esechie *et al.* 1977). In this study the presence of brace roots showed significantly positive correlation with other important agronomic characters, which is partially in accordance with the previous study. However, more research needs to be done to analyze the relationship between the number of nodes with brace roots, the brace root number on each node and other traits, like plant height, stem diameter, puncture strength and stem pulling force, to find lodging resistance related characteristics.

To date, the numbers, angles and branching density of brace roots have not yet been used for the investigation of root architecture in sorghum. Recently identified sorghum root angle QTL are co-located with previously identified QTL for stay-green in sorghum and associated with grain yield (Mace *et al.* 2012). The angles and branching are identified to be consistent between brace and crown roots in maize (Trachsel *et al.* 2011). Thus the identification of brace root QTLs in this study, together with the identification of nodal root angle QTL, may present new opportunities for improving drought adaptation and grain yield via molecular breeding to manipulate the root traits for which selection has previously been very difficult.

Due to the difficulty and complexity of measuring root traits in the field, previous studies on plant root traits have mainly concentrated on primary root and seminal root characteristics, which further hindered the identification of the relevant genetic loci in such conditions. In sorghum, a few studies have presented evidence of genotypic variation for root traits (Bhan *et al.* 1973, Blum *et al.* 1977, Mayaki *et al.* 1976). Recently, four QTLs for nodal root angle (*qRA1_5*, *qRA2_5*, *qRA1_8*, *qRA1_10*), three QTLs for root dry weight (*qRDW1_2*, *qRDW1_5*, *qRDW1_8*) and eight QTLs for root volume, root fresh weight and root dry weight were identified by Mace *et al.* (2012) and Rajkumar *et al.* (2013), respectively. This study aimed to characterize the inheritance pattern of brace roots and to map the genetic loci controlling the traits. The results demonstrated that the presence and absence of brace roots are independent of the other root architecture traits measured by other studies. To our knowledge, this is the first report in which brace root trait controlling loci were localized in sorghum, which provides useful information for investigating the molecular mechanisms that

underline the sorghum root system. However, this study was a primary investigation of presence or absence of brace roots in sorghum. With the construction of recombinant inbred lines (RIL), fine mapping of the two QTLs is underway. In addition, we are trying to quantify the role played by brace roots, which could help us to accurately map the loci and find possible candidate genes in the major QTL region and finally clone the gene. The brace roots provide anchorage to the plant, which is a key factor for plant root lodging resistance. In maize, high plant density is regarded as one of the most important goals for improving grain yield. Root lodging resistance selection has played a dominant role in driving the historical increase (c. four-fold) in plant density in the past 50 years (Ku *et al.* 2012). The characterization and identification of the two QTLs, therefore, could facilitate marker-assisted selection for root architecture improvement to create hybrids with strong plant body stability in sorghum breeding programs.

For the past two decades, family-based mapping population from biparental crossing has been employed for mapping root-related trait loci (de Dorlodot *et al.* 2007). Recently, association or linkage disequilibrium mapping has become a popular method for dissecting the genetic basis of complex traits in plants and has been successfully applied in sorghum (Lv *et al.* 2013, Morris *et al.* 2013). Compared with traditional family-based mapping population, association mapping can investigate hundreds of varieties in one natural population. In addition, naturally occurring recombination will have occurred over many generations and consequently linkage blocks will be substantially smaller in an association mapping population, thus enabling more fine-scale mapping of genes controlling traits. Therefore, an association study should be conducted to survey more germplasm to find more accurate loci controlling the presence and number of brace roots. As it is now relatively easy to genotype large numbers of varieties, the next rate-limiting step will be the development of high-throughput root phenotyping systems under laboratory or field conditions. Also, several studies on plant primary and lateral root transcript profiles using deep sequencing have been reported (Fizames *et al.* 2004, Li *et al.* 2011, Poroyko *et al.* 2005), which could help investigate their regulating mechanisms and identify transcripts involved in development of plant root systems, including brace roots.

Acknowledgement

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